American Journal of Pathology, Vol. 161, No. 6, December 2002

Short Communication

Craniopharyngiomas of Adamantinomatous Type Harbor *β-Catenin* Gene Mutations

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Craniopharyngioma is a rare tumor occurring in the sellar region comprising 3% of all intracranial tumors. To elucidate the contribution of *β-catenin* gene mutation to tumorigenesis, we examined genetic alterations and expression of β-catenin in 10 cases of adamantinomatous and 6 cases of papillary craniopharyngiomas. *β-Catenin* gene mutations were found in all of the adamantinomatous and none of the papillary craniopharyngiomas. Immunohistochemically, all cases of adamantinomatous craniopharyngioma showed cytoplasmic and nuclear expression of β-catenin. In contrast, papillary craniopharyngiomas showed exclusively membranous expression. The results suggest that adamantinomatous- and papillary-type craniopharyngiomas are not only clinicopathologically, but also genetically, distinctive variants. Mutation of the *β-catenin* gene therefore seems to play an important role in the tumorigenesis of adamantinomatous craniopharyngioma. Among the adamantinomatous-type tumors, β-catenin-positive mesenchymal cells were observed in two cases. Microdissection-based mutational analysis revealed that these mesenchymal cells also harbor the same *β-catenin* gene mutations as those of epithelial cells, suggesting their tumorous nature. Thus, at least a subset of adamantinomatous craniopharyngioma is considered to be biphasic. *(Am J Pathol 2002, 161:1997–2001)*

Craniopharyngioma is a rare neoplasm occurring in the sellar region, comprising 3% of all intracranial tumors.[1] Although its origin is not firmly established, it is generally thought to be derived from remnants of Rathke's pouch. Craniopharyngioma consists of two subtypes: adamantinomatous and papillary. Traditional descriptions of craniopharyngioma have focused on the adamantinomatous type, characterized histologically by a complex epithelial pattern with lobules, cystic spaces, and peripherally palisading cells. Keratin nodules (wet keratin) and calcifications are well-recognized diagnostic hallmarks. The papillary type is an increasingly recognized variant, composed of papillary squamous epithelium lacking features of classical adamantinomatous craniopharyngioma. These two variants are considered to be distinct not only histologically, but also clinically. Papillary craniopharyngioma is reported to occur almost exclusively in adults,[2–5] whereas adamantinomatous craniopharyngioma has a broader age distribution with a predilection for childhood and early adolescence.[3–5]

Genetic alterations in craniopharyngioma is poorly understood. Although multiple chromosomal abnormalities have been reported in two cases,[6,7] no specific genetic alterations have been described so far. β-Catenin is a submembranous component of the adherence junction, and it also acts as a transcriptional activator of the Wnt signaling pathway. Mutations of the *β-catenin* gene have been reported in various tumors.[8] These genetic alterations result in stabilization of β-catenin and up-regulation of Tcf/Lef-dependent transcriptional activity.[8,9] In the present study, we analyzed mutation of the *β-catenin* gene and β-catenin expression to elucidate their contribution to the tumorigenesis of craniopharyngiomas.

Materials and Methods

Nineteen surgically resected craniopharyngiomas obtained from 16 patients were examined in the present study. These included 10 cases of the adamantinomatous type and 6 cases of the papillary type. The samples

Supported in part by a Grant-in-Aid for Second Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor, and Welfare, Japan.

Accepted for publication August 29, 2002.

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were routinely fixed with 10% formalin and embedded in paraffin.

Five-μm-thick sections of each specimen were stained briefly with hematoxylin and eosin and subjected to DNA extraction. The tumorous areas were dissected using sterilized toothpicks under a microscope. Nontumor samples were available in only three cases (cases 1, 3, and 10), in which enough amount of glial tissue was obtained. The dissected samples were incubated in 30 μl of DNA extraction buffer (50 mmol/L Tris-HCl, pH 8.0, 1 mmol/L ethylenediaminetetraacetic acid, 0.5% (v/v) Tween 20, 200 μg/ml proteinase K) at 37°C overnight. Proteinase K was inactivated by heating at 100°C for 10 minutes. The samples were subjected to polymerase chain reaction (PCR) with a previously described pair of primers encompassing glycogen synthase kinase-3β (GSK-3β)-phosphorylation sites of the *β-catenin* gene, CT-S-F (5′-ATGGAACCAGACAGAAAAGCG-3′) and CT-S-R (5′-CAGGATTGCCTTTACCACTCA-3′).[10] PCR was performed for 3 minutes at 95°C for initial denaturing, followed by 40 cycles at 94°C for 15 seconds, 58°C for 30 seconds, and 72°C for 60 seconds, and a final extension at 72°C for 5 minutes. The PCR products were electrophoresed in a 2% (w/v) agarose gel, visualized under UV light with ethidium bromide staining, and recovered using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Isolated PCR products were sequenced on an Applied Biosystems 310 Genetic Analyzer (Applied Biosystems Inc., Foster, CA). Each experiment was done at least two times, including DNA extraction.

Two cases suspected to have a mesenchymal component were further subjected to laser capture microdissection-based analysis as described previously.[11] The epithelial and mesenchymal components were separately microdissected using an LM200 laser capture microdissection system (Arcturus, Mountain View, CA). Microdissection was performed using peripherally palisading cells as a hallmark of borders between the epithelial and mesenchymal cells. The dissected samples were incubated in 20 μl of DNA extraction buffer and analyzed by PCR followed by direct sequencing.

Immunohistochemical staining was performed by the avidin-biotin complex method. The primary antibody used was monoclonal anti-β-catenin (C19220, 1:200 dilution; Transduction Laboratories, Lexington, KY). 3–3′-Diaminobenzidine tetrahydrochloride was used as a chromogen.

Results

All adamantinomatous craniopharyngiomas showed a complex epithelial pattern with peripherally palisading cells and wet keratin (Figure 1A). Calcification, foreign body giant cells, and cholesterin clefts were frequently observed. Papillary craniopharyngiomas showed stratified squamous epithelium-lined papillae with loose fibrovascular cores (Figure 1B). Characteristic features of the adamantinomatous variant, such as wet keratin, palisading cells, and calcification, were absent. Nuclear atypia was absent in both variants. There were no cases showing mixed features.

Sequencing analysis revealed *β-catenin* gene mutations in all 10 adamantinomatous craniopharyngiomas, whereas none were present in the 6 papillary craniopharyngiomas analyzed (Table 1). All of the mutations were missense mutations affecting the serine/threonine residues at GSK-3β phosphorylation sites or an amino acid flanking the first serine residue. The mutational status of two recurrent cases was concordant in each tumor. Three nontumor samples examined showed no genetic alterations.

Immunohistochemistry for β-catenin showed quite different results between the two subtypes. All cases of the adamantinomatous type showed cytoplasmic and nuclear accumulation (Figure 1C). Sheets of epithelial cells showed weak to moderate cytoplasmic and membranous staining. Peripherally palisading cells showed somewhat stronger expression in both the cytoplasm and membrane. Clusters of cells showing strong nuclear accumulation were seen among the whorl-like arrays of the epithelial cells, where present. In cases of the papillary type, tumor cells showed exclusively membranous staining (Figure 1D).

Among the 10 cases of adamantinomatous craniopharyngioma, 2 showed distinctive features in the stroma surrounding the epithelial cell nests. These were whorling and streaming fascicles of spindle cells around the epithelial tumor cell nests (Figure 2, A and B). In case 8, these spindle cells formed fascicles of variable cellularity, and surrounded most of the epithelial cell nests (Figure 2A). In case 1, these spindle cell fascicles were less cellular (Figure 2B), and distributed only focally. They were absent at the invasive front, where the epithelial tumor nests had infiltrated the brain tissue. The stroma of these cases was more cellular than those of the other tumors even in case 1, and the streaming arrangement of stromal cells was distinctive in these two cases. In sections stained immunohistochemically for β-catenin, these spindle cells exhibited moderate to strong cytoplasmic and nuclear staining and lacked the membranous staining observed in epithelial tumor cell nests (Figure 2, C and D). β-Catenin expression was markedly stronger in highly cellular areas in case 8 (Figure 2C).

We suspected the tumorous nature of these mesenchymal spindle cells, and performed laser capture microdissection to determine whether mesenchymal cells overexpressing β-catenin also harbor *β-catenin* gene mutations. Epithelial cell nests and mesenchymal cells were separately microdissected and subjected to PCR followed by direct sequencing (Figure 3, A and B). This revealed the same mutations in both the epithelial and mesenchymal components (Figure 3C). The analysis was repeated three times, using a different area of a different sample each time, and we obtained identical results in every experiment. In case 1, nonneoplastic glial tissue was examined and showed no mutation as described above, indicating an absence of germ line mutation.

Discussion

β-Catenin gene mutations were found in all adamantinomatous craniopharyngiomas. All mutations caused amino

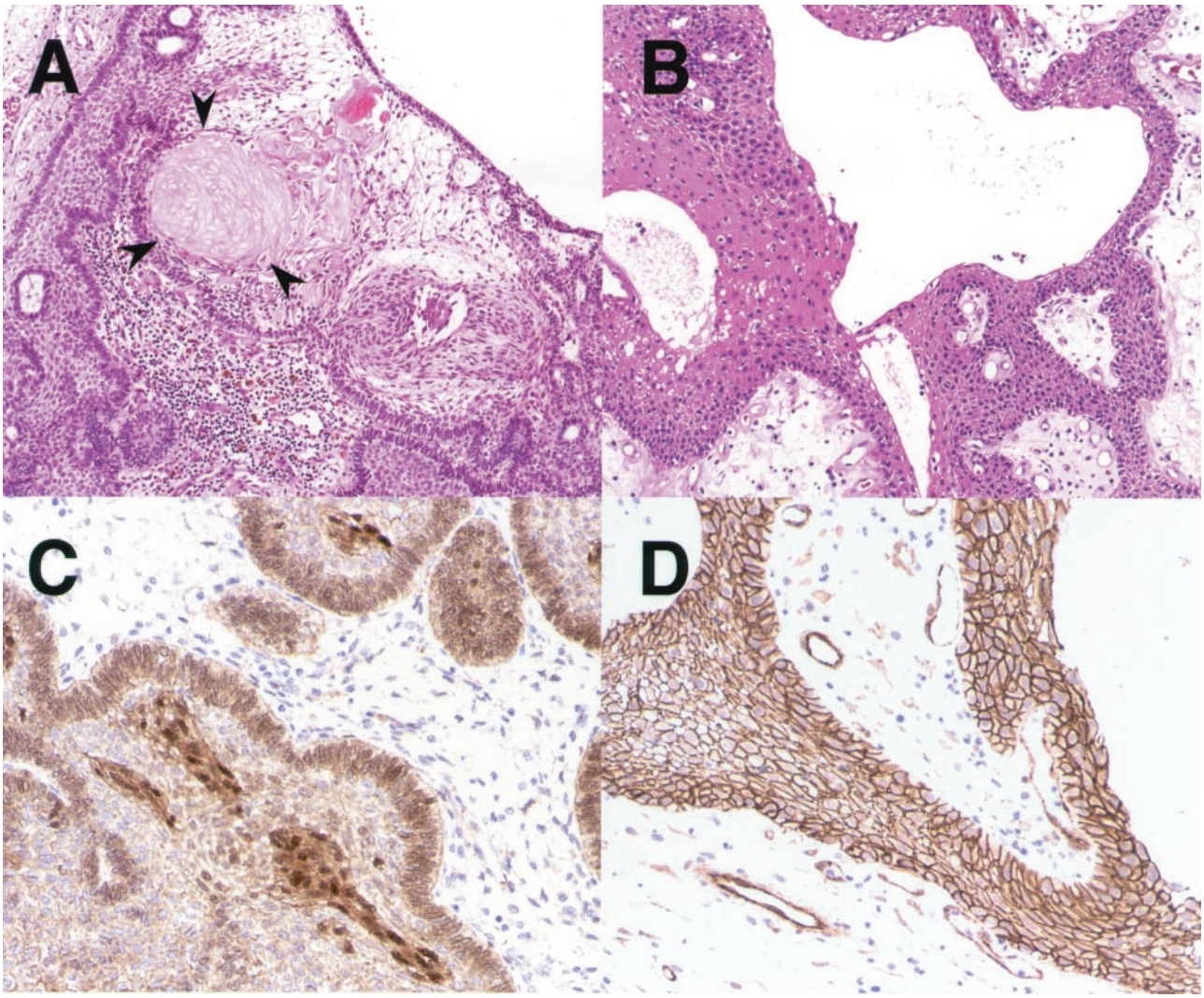

Figure 1. Representative histology of cases (**A**, **B**) and immunohistochemistry for β-catenin (**C**, **D**). **A:** Adamantinomatous craniopharyngioma. Loosely cohesive squamous cells surrounded by peripherally palisading cells. A squamous nodule (wet keratin) (**arrowheads**) is observed (case 7-1). **B:** Papillary craniopharyngioma. Well-differentiated squamous epithelium lining loose connective tissue (case 16). **C:** Adamantinomatous craniopharyngioma. Membranous and cytoplasmic staining for β-catenin. Peripherally palisading cells show stronger expression. Clusters of cells along the epithelial cell whorls show strong nuclear accumulation (case 7-1). **D:** Papillary craniopharyngioma. Membranous staining pattern (case 11). H&E (**A**, **B**) and immunohistochemistry for β-catenin (**C**, **D**). Original magnifications: ×100 (**A**, **B**); ×200 (**C**, **D**).

acid substitution of GSK-3β phosphorylation sites or of a residue flanking one of these phosphorylation sites, similar to the mutations that have been reported previously in other tumors.[8] These mutations are considered to cause β-catenin stabilization by inhibiting phosphorylation by GSK-3β and subsequent proteosomal degradation. Consistently, all of the cases of the adamantinomatous craniopharyngiomas showed nuclear and cytoplasmic accumulation of β-catenin. In contrast, none of the papillary-type tumors harbored mutations, and all of them showed an exclusively membranous staining pattern as usually seen in nonneoplastic epithelial cells. *β-Catenin* gene mutations and overexpression of β-catenin are considered to be characteristics of adamantinomatous craniopharyngiomas. Therefore, constitutive activation of Tcf/Lef-dependent transcription by β-catenin stabilization seems to be deeply involved in the tumorigenesis of these neoplasms.

Although clinicopathological differences between the two variants have been reported,[2–5] there has also been some debate about the need for this subdivision. Because craniopharyngioma is a complex epithelial tumor exhibiting a variable histological appearance, such a subdivision would only serve to reiterate the heterogeneity of these tumors.[1] The presence of mixed variants showing transitional features between the adamantinomatous and papillary variants has also been described.[4,5] However, in the present study, mutations of the *β-catenin* gene and expression of β-catenin appeared to clearly discriminate these two subtypes. Examination of β-catenin status in cases showing transitional features would help to certify their identity and understand the relationship of the two variants.

Previously craniopharyngiomas have been undoubtedly regarded as epithelial tumors. Unexpectedly, however, 2 of the present 10 adamantinomatous craniopharyngiomas

Table 1. Results of Immunohistochemical and Mutational Analysis

Case	Age/sex	Type	Cytoplasmic/ nuclear staining	Affected codon	Mutation
1	4/M	Adamantinomatous	+	33	TCT(Ser)>TTT(Phe)
2	4/M	Adamantinomatous	+	33	TCT(Ser)>TTT(Phe)
3	5/M	Adamantinomatous	+	33	TCT(Ser)>TTT(Phe)
4	12/M	Adamantinomatous	+	33	TCT(Ser)>CCT(Pro)
5	35/M	Adamantinomatous	+	33	TCT(Ser)>TGT(Cys)
6	36/F	Adamantinomatous	+	32	GAC(Asp)>GGC(Gly)
7–1	38/M	Adamantinomatous	+	37	TCT(Ser)>TTT(Phe)
7–2	39/M	Adamantinomatous	+	37	TCT(Ser)>TTT(Phe)
8	45/M	Adamantinomatous	+	41	ACC(Thr)>GCC(Ala)
9	46/M	Adamantinomatous	+	32	GAC(Asp)>CAC(His)
10	54/F	Adamantinomatous	+	37	TCT(Ser)>TTT(Phe)
11	24/M	Papillary	–	–	
12	46/M	Papillary	–	–	
13–1	47/M	Papillary	–	–	
13–2	50/M	Papillary	–	–	
13–3	57/M	Papillary	–	–	
14	55/F	Papillary	–	–	
15	55/F	Papillary	–	–	
16	58/M	Papillary	–	–	

were shown to have a mesenchymal component by genetic and immunohistochemical analysis. One explanation for these tumors is that they belong to a distinctive subtype, which could be named "craniopharyngioma-fibroma." However, it is also possible that adamantinomatous craniopharyngiomas are not infrequently biphasic, and that the presence of mesenchymal components has simply been overlooked. Without immunohistochemical staining, the bi-

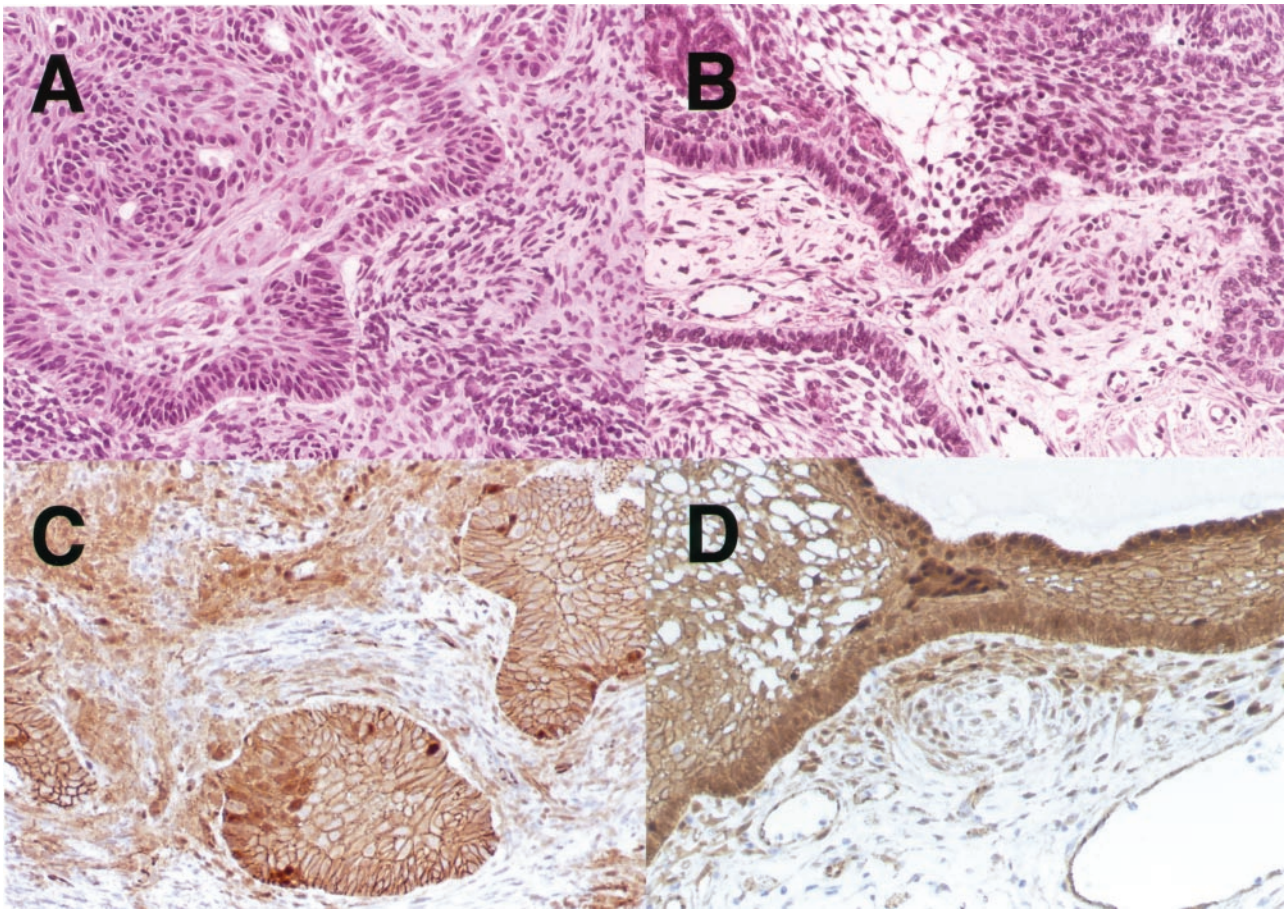

Figure 2. Adamantinomatous craniopharyngioma with a mesenchymal component (**A**, **C**: case 8; **B**, **D**: case 1). **A** and **B:** Whorling and streaming spindle-cell fascicles are observed around the epithelial cell nests. A highly cellular area is seen in case 8 (**A**). **C** and **D:** Immunohistochemistry for β-catenin. Spindle cells around the epithelial cell nests show moderate to strong cytoplasmic and nuclear expression and lack membranous staining observed in epithelial cells. H&E (**A**, **B**) and immunohistochemistry for β-catenin (**C**, **D**). Original magnifications, ×200.

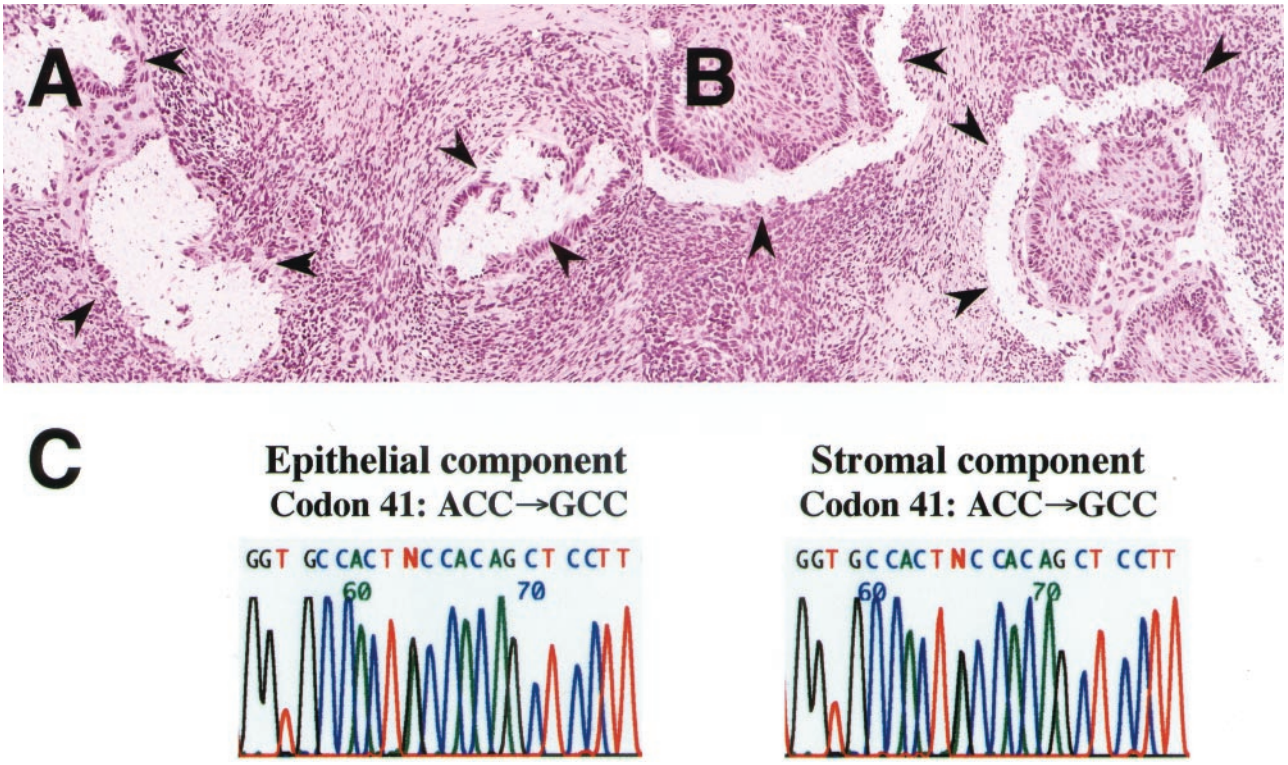

Figure 3. Microdissection-based analysis (case 8). **A** and **B:** Sections after microdissection for epithelial (**A**) and mesenchymal (**B**) components. Microdissection was performed using peripherally palisading cells as a hallmark of borders between the epithelial and mesenchymal cells. Microdissected areas were marked by **arrowheads**. **C:** Direct sequencing of the *β-catenin* gene. Both components harbor missense mutations affecting a threonine residue at GSK-3β phosphorylation sites. H&E (**A**, **B**). Original magnifications, ×200 (**A**, **B**).

phasic nature of these cases might have remained obscure. Moreover, the mesenchymal component appeared to be present only focally, as in case 3. Re-evaluation for the presence of the mesenchymal components in adamantinomatous craniopharyngiomas may be required to decide where to place these biphasic tumors and it would also provide some clues to understand their histogenesis.

In the present study, we showed that *β-catenin* gene mutations and β-catenin overexpression in craniopharyngiomas were frequently and exclusively present in the adamantinomatous variant. The results suggested that adamantinomatous and papillary craniopharyngiomas are distinctive not only histologically and clinically, but also genetically. Our present findings also show that at least a subset of adamantinomatous craniopharyngiomas have a mesenchymal component, ie, they are biphasic. Further investigation will be required to determine the significance of biphasic craniopharyngiomas.

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